

ENU F2 Mutagenesis – Day 1

- Prepare ENU stock solutions in fresh 100% ethanol (40 mM = 6.5 mg/ml (it's 40% water! **WEAR GLOVES AND PROPERLY DISPOSE OF ALL WASTE!**)
 - If necessary, the precise amount of ENU in a solution can be ascertained by measuring the optical density of the solution at 398 nm (1 mg/ml = 0.72. OD)
- Dilute stock ENU to 2.4 mM in 1 ml M9 buffer (60µl 40 mM ENU + 940 µl M9).
- Rinse L4 stage worms off the plate; rinse 1-2X with M9 to get rid of bacteria.
- Resuspend worms in 3ml M9 in 15 ml conical tube.
- Add the 1ml of 2.4 mM ENU to the worms (Final volume 4 ml; final ENU concentration - 0.6 mM)
- Mutagenesis is done at room temperature for 4 h with gentle agitation in a fume hood (ie, 50 rpm on a rotary shaker).
- Following mutagenesis, worms are washed 5X with M9 buffer and placed on NGM plates for 24 hours.
- **All ENU solutions should be rendered inactive before being discarded. Solutions can be brought to a final concentration of 1% sodium hydroxide and kept at room temperature for 1 h (6 half-lives).**

Day 2

- 24 hours after ENU mutagenesis, pick 5 gravid adult P0s to 10 NGM 10cm plates. After 3 hours, move the P0s to 10 new NA22 10cm plate. Do this again after another 3 hours (3 sets of 10 NGM plates – 30 plates total)
- Put one batch of 20 plates at 20°. Put the other 2 sets at 16°.

Day 3

- 1.5 days after ENU exposure, move the second set of plates to 20°.

Day 4

- 2.5 days after ENU exposure move the third set of plates to 20°.

Day 5

- Check to see if F2 eggs have started hatching. If so, count the total # of F1 adults on 1-several plates (depending on how uniform the plates are). This allows you to calculate the # of haploid genomes screened (Total # of F1s X 2 for an F2 screen).

Day 6

- Count F1s today if you didn't do it yesterday. Make sure you get reasonable counts from the B and C plates, since they are different.

Day 7

OFF

Day 8

- Examine F2 adults from 1st set of 20 plates for phenotype (lack of GFP expression in *osm-11; kbls5*).
- Clone individual mutants (as many as possible) into single well of a 12 or 24 well plate
- Note which plate each candidate comes from – you can only keep 1 candidate / plate to ensure independence of events
- Put candidates at 20°

Day 9

Examine 2nd set of plates and clone mutants as before
Reexamine 1st set of plates to see if you missed anything

Day 10

Examine 3rd set of plates and clone mutants as before
Reexamine 2nd set of plates

Day 11

Reexamine 3rd set of plates
Start looking at progeny of candidates to determine which ones breed true